

**iGEM TU/e 2014**

Biomedical Engineering

Eindhoven University of Technology  
Room: Ceres 0.04  
Den Dolech 2, 5612 AZ Eindhoven  
The Netherlands  
Tel. no. +31 50 247 55 59  
[2014.igem.org/Team:TU\\_Eindhoven](http://2014.igem.org/Team:TU_Eindhoven)

**Date**

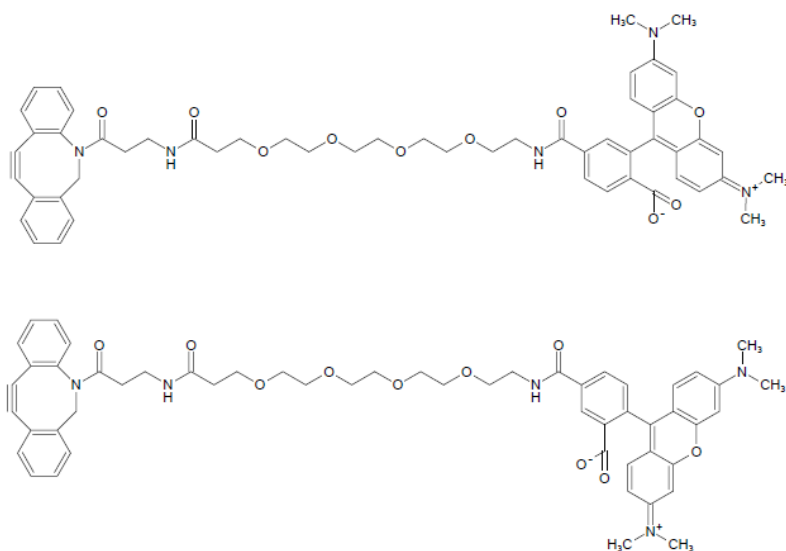
11 August 2014

# **FACS - DBCO-PEG<sub>4</sub>-5/6-TAMRA**

## Table of contents

<b>Title</b>	<b>1</b>	<b>Stock solutions</b>	<b>3</b>
FACS - DBCO-PEG4-5/6-TAMRA	<b>2</b>	<b>Preparation of FACS samples</b>	<b>3</b>

# 1 Stock solutions



DBCO-PEG<sub>4</sub>-5/6-TAMRA, molecular weight: 936.06 g/mol;  $\lambda_{abs}$  545 nm;  $\lambda_{em}$  565 nm

- 5 mM DBCO-PEG<sub>4</sub>-5/6-TAMRA
- 833  $\mu$ M DBCO-PEG<sub>4</sub>-TAMRA
- Buffer: PBS

# 2 Preparation of FACS samples

- Prepare following tubes:

Tube	[DBCO]	Buffer volume to add	Cells (10 <sup>8</sup> )	DBCO volume to add ( $\mu$ L)		DBCO/tag ratio
				5 mM	833 $\mu$ M	
1	0	150 $\mu$ L	50 $\mu$ L			
2	30 $\mu$ M	0 $\mu$ L	200 $\mu$ L	1.21		1,1E+04
3	5 $\mu$ M	0 $\mu$ L	200 $\mu$ L		1.21	1,8E+03
4	30 $\mu$ M	100 $\mu$ L	100 $\mu$ L	1.21		2,2E+04
5	5 $\mu$ M	100 $\mu$ L	100 $\mu$ L		1.21	3,6E+03
6	30 $\mu$ M	150 $\mu$ L	50 $\mu$ L	1.21		4,4E+04
7	5 $\mu$ M	150 $\mu$ L	50 $\mu$ L		1.21	7,3E+03

- React DBCO tubes for 1h to 6h in shaking block at 4°C
- Prepare FACS samples after 1 and 6 hours:
  - Spin down the cells for 10 min at 13,400 rpm
  - Resuspend with 1 mL ice cold PBS
  - Spin down the cells for 10 min at 13,400 rpm and put on ice until FACS
  - Right before FACS: resuspend with 1 mL PBS